Customer case study
Field of study: Hematology

In Sickle Cell Project, Baylor Scientist Discovers Link to Fetal Hemoglobin Production

When Baylor's Vivien Sheehan won a grant competition to use QIAGEN's Ingenuity Variant Analysis, she found an exciting association in sickle cell anemia patients. It explains how a current drug works and opens the door for a possible new drug target to boot

Just two decades ago, there were virtually no therapeutic treatments to manage hemoglobin production for patients with sickle cell anemia, a severe condition in which blood cells do not carry enough oxygen to support the body. Today, there's not only a medication, but thanks to new research from Vivien Sheehan and data interpretation from QIAGEN's Ingenuity Variant Analysis, scientists also have a better understanding of how this drug works — as well as a promising new lead for another treatment option.

Sheehan, an Assistant Professor of Pediatrics at Texas Children's Hematology Center and Baylor College of Medicine, won a corporate grant to use Ingenuity Variant Analysis for a project studying fetal hemoglobin response in children treated with hydroxyurea, a chemotherapy agent approved in the '90s for low-dose use in sickle cell patients. Sheehan's goal was to use the genomics interpretation platform to mine exome sequence data for genetic variants related to drug response.

Now, Sheehan has crunched data from nearly 180 exomes. While a linear association turned up no useful results, data interpretation with Ingenuity Variant Analysis was able to direct Sheehan to a gene called FOXO3 that appears to be linked to a patient's response to hydroxyurea. In addition, the Ingenuity Variant Analysis helped steer Sheehan away from a number of red herrings, saving valuable time and focusing her attention on the findings most likely to matter. She followed up with extensive functional studies and confirmed the biological effect of FOXO3 mutations.

The Sickle Cell Focus

Sheehan didn't start out with a plan to specialize in sickle cell disease. She earned her PhD in biochemistry at Texas A&M University from a lab she first joined through a school job that had her washing dishes and glassware. It was her first real exposure to the world of research, and set Sheehan on her career path in biology. Afterward, she headed to medical school at Emory University, where she found herself drawn toward hematology because the diseases were so molecular in nature. "It was a lot like the work I'd done for my PhD," she says.

But during her time in medical school, she realized that her original area of interest — tumorigenic viruses — was the focus of countless researchers. Sickle cell disease, which was fairly common in the area near Emory, did not get that kind of attention. "Seeing how complex the disease was, and how varied its presentation was, really fascinated me," she recalls. "The fact that there weren't a lot of people with PhDs doing research in this area made me think I could make a difference."

Indeed, patients diagnosed with sickle cell disease need all the advocates they can get. In the developed world, where patients are identified early and treated with prophylactic antibiotics, life expectancy tops out at 45 years. In other regions of the world, patients usually don't live past the age of 5. A drug that was approved some 20 years ago for sickle cell use, hydroxyurea, is seen as the best hope for increasing lifespan for patients, but its mechanism has been largely unknown.

Today, Sheehan has her own lab at Baylor and also sees patients at Texas Children's Hospital. She's able to translate much of her research into better patient care, focusing on teenage and adult patients who have fewer choices of providers. "Sickle cell really does change as you go from pediatric to adult care," Sheehan says. In one project, she's working with a subset of sickle cell patients with a milder phenotype who have been excluded from clinical trials. There are no clear treatment guidelines for them, so Sheehan aims to establish a specific clinical protocol for this group of patients.

Finding FOXO3

When Sheehan landed at Baylor after her fellowship at St. Jude Children's Research Hospital, she suddenly had access to a remarkable resource: the school's Human Genome Sequencing Center.

Together with other scientists, she embarked on an effort to sequence the exomes of nearly 180 patients from two pediatric sickle cell clinical trials. The goal was to learn more about drug mechanism and response for hydroxyurea, which helps patients produce fetal hemoglobin, a protein that most people stop producing after infancy. Because fetal hemoglobin doesn't sickle, its presence in the bloodstream can ameliorate effects of the sickle cell disease. Sheehan and other scientists were looking for variants associated with fetal hemoglobin response to the therapeutic.

Thanks to a tip from her mentor, Eric Boerwinkle, Sheehan also decided to look at baseline data. "We didn't find anything with linear association," she says. But Ingenuity Variant Analysis lets users perform burden analysis, and "in doing that we found that there were variants in a gene called FOXO3 that were associated with a lower baseline fetal hemoglobin," she adds.

The gene hadn't come up in previous genome-wide association studies of the disease; it was the deep dive in Sheehan's study that allowed her to find the link. "You can't do a burden analysis unless you have the detail of whole exome or whole genome sequencing," she says.

Despite the novelty of the finding, some scientists worried that the variant in FOXO3 was low-frequency and therefore not very useful. But Sheehan persisted. "I went ahead and did the functional studies on it." These included knocking FOXO3 down in an erythroid cell line that produces fetal hemoglobin as well as in primary erythroid cells. "Both of those models recapitulated the patient phenotype: when we knocked down FOXO3, it decreased fetal hemoglobin," Sheehan says. She also overexpressed FOXO3 in the model systems and found a marked increase in hemoglobin.

The FOXO3 research continues, particularly because the gene is already targeted by therapeutics on the market. "FOXO3 potentially could be a drug target," Sheehan says. "There are some quite benign medications that have been shown to upregulate FOXO3, such as resveratrol and more potent antioxidants." While still years away from potential

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clinical use, the finding offers a new path that might one day help ease symptoms for sickle cell patients.

And Sheehan wouldn't have discovered it without Ingenuity Variant Analysis, which she gained access to by winning a grant program offered by the company. "Getting the grant to be able to use it was really helpful," she says, noting that she has no background in statistics. "With all this data, you really need a great statistician or a program like Ingenuity Variant Analysis that's very user-friendly," she adds.

The application proved easy to learn for Sheehan, who was eager to conduct her own analysis on the large exome data set. A feature that she found particularly useful was the Confidence filter and its ability to flag or block genes that are highly variable and unlikely to be drivers in a data set. "There are a few genes I would've pursued, but seeing that they're on the list of usual suspects helped guide me to other results," Sheehan says. "Later investigation in the literature showed that those genes really were dead ends."

Sheehan says she also appreciates the flexibility of Ingenuity Variant Analysis. "It's great that it allows you to do a gene-based approach as well as a variant-based approach," she says. "I really have enjoyed using it."

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